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The production of volatile organic compounds during nitrogen transformations in soils

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Abstract

The volatile organic compounds produced during a sequence of soil incubations under controlled conditions, with either added NH_4^+-N or NO_3^--N, were collected and identified. The nature and relative amounts of the volatile organic compounds produced by the microorganisms in the soils were remarkably reproducible and consistent.

Introduction

There is a large variety of volatile organic compounds (VOCs), in a wide range of concentrations, in soil atmospheres. These VOCs may come from either internal sources such as plants or microorganisms, or external sources as soil is a major sink for many atmospheric constituents.

Most VOCs in atmospheres are probably microbial in origin (Stotzky and Schenck, 1976). So, production of them will probably be influenced by factors that influence either population dynamics or microbial activities.

Total soil microbial biomass is relatively stable throughout the growth of crops (Ritz et al., 1992), while specific activities such as nitrification and denitrification show very dynamic temporal changes in rates (Wheatley and Williams, 1989; Wheatley et al., 1991). These changes in activity rates can be both widespread and rapid throughout the bulk soil. Variations in the pattern of VOC production may reflect the type and amount of activity occurring in soils, and also changes in cultural conditions. These compounds may also have a role in microbial communication in the rhizosphere.

Materials and methods

Soil

Samples were taken from a field of Carbrook association soil cropped to potatoes. The soil was an estuarine silty-clay loam; pH 6.1, total C 2.23% (w/w), total N 0.22% (w/w) and water-holding capacity (WHC) 0.47 mL H_2O g^{-1} dry soil. P at a rate equivalent to 85 kg ha^{-1} and K at 220 kg ha^{-1}, had been applied to the soil. No N was applied to these plots at any time.

Soil incubation and volatile product collection

After sieving, <4 mm, 2.5 kg fresh soil was placed in each of triplicate 5 L quickfit vessels, then amended with water and appropriate solutions. Soya broth (Oxoid, Ltd.; 250 mL) was added to all the treatments, and 25 mL of a 25 g L^{-1} solution of NH_4NO_3 and 25 mL of glucose, 30% (w/v) to one set and 25 mL $(NH_4)_2SO_4$, 0.3% (w/v) to another. The third received no further amendments. Water to 50% of the WHC was added to all. Air and O_2-free N_2 were used alternatively, for 24 h periods, to purge the headspace products from the soils through a tube of adsorbant, 0.4 g Haysep Q, 800–100 mesh,(Analytical Polymers Inc., Bandera, Texas). Then the tube was removed and dried by reverse flushing with O_2-free N_2 at 20 mL min^{-1} for 10 min. All gases used in the incubations were passed through filters of molecular sieve and activated

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charcoal that had been previously conditioned in a He stream, 25 mL min^{-1}, for 24 h at 180 °C, then a sterile sintered-steel filter, before use. Similarly the collection tubes were conditioned in a He stream, 10 mL min^{-1}, for 24 h at 180°C.

Analyses of the volatile organic compounds produced

The VOCs adsorbed by the porous polymer were analysed on an integrated system consisting of a Perkin Elmer ATD50 automated thermal desorber connected to a Hewlett Packard 5890 gas chromatograph interfaced to a VG Trio 1000 quadrupole mass spectrometer. The sample tubes were desorbed at 130 °C for 15 min with an outlet split ratio of 7.5:1. The mixture was then separated on a DB1701 chromatographic column, 60 m long by 0.25 mm i.d., 1 μm film, utilising He at 1 mL min^{-1} as the carrier gas. The oven was temperature programmed from 40 °C to 240 °C at 5 °C min^{-1}, final isothermal period 20 mins. Compounds were identified by retention time and comparison of the mass spectra with standards or published mass spectral data bases.

Compounds in the C_3 to C_{10} range could be identified in this study. The relative proportions of such VOCs produced by soil micro-organisms, under both aerobic and anaerobic conditions, were then determined.

Table 1. Volatile organic compounds detected in the headspace of aerobically and anaerobically incubated soil

Alcohols	**Ketones**
Ethanol	Propan-2-one
Propan-1-ol	Butan-2-one
Propan-2-ol	Pentan-2-one
Butan-1-ol	Pentan-3-one
Butan-2-ol	4-Methyl pentan-2-one
2-Methyl propan-1-ol	5-Methyl heptan-2-one
2-Methyl butan-1-ol	3-Hydroxy butan-2-one
3-Methyl butan-1-ol	
Aldehydes	**Aromatics**
2-Methyl-butan-1-al	Benzene
3-Methyl-butan-1-al	Ethyl benzene
Sulphides	Dimethyl benzene
Dimethyl sulphide	Methylethyl benzene
Dimethyl disulphide	Trimethyl benzene
Dimethyl trisulphide	Benzaldehyde
2-Methyl propylsulphide	
Methyl Esters	**Ethyl Esters**
2-Methyl butanoic acid	Acetic acid
3-Methyl-butanoic acid	Butanoic acid
Butyl Esters	2-Methyl propanoic acid
Acetic acid	2-Methyl-butanoic acid
	3-Methyl-butanoic acid

Results

A total of 35 VOCs were collected and identified from the soil samples. The range of compounds detected (Table 1) included aliphatic alcohols, aldehydes, ketones and esters, polysulphides and variously substituted simple aromatic compounds. The production of these VOCs in each of the soils studied was reproducible under defined conditions, both with respect to the compounds identified and their relative proportions.

Aerobic incubations

The predominant compounds produced under aerobic conditions were sulphides, accounting for more than 65% of the total VOCs in all incubations (Table 2). The most abundant S-compound was dimethyl disulphide, representing over 90% of the total sulphides identified. Smaller quantities of dimethyl sulphide and dimethyl trisulphide were also present in the aerobic incubations and the relative proportions of each did not vary with amendment.

The relative proportions of volatile ketones were significantly increased by the addition of either KNO_3 or $(NH_4)_2SO_4$. In all but the $(NH_4)_2SO_4$-enriched incubations only 2 ketones were detected, propan-2-one and butan-2-one, the latter predominated in all cases. In the $(NH_4)_2SO_4$-enriched incubations trace amounts of both pentan-2-one and 4-methyl pentan-2-one were also identified.

Adding $(NH_4)_2SO_4$ also significantly increased the proportion of aldehydes, and uniquely under aerobic conditions produced 2-methyl butan-1-al. 3-methyl butan-1-al was the predominant aldehyde in the $(NH_4)_2SO_4$-enriched incubation and the only aldehyde detected in the 3 other aerobic treatments.

An increase in the amount of ethanol, the predominant alcohol in all the aerobic treatments, caused a significant increase in the proportion of alcohols produced (Table 2) when $(NH_4)_2SO_4$ was added. However, other alcohols including propan-2-ol, 2-methy propan-1-ol, butan-1-ol and 3-methyl butan-1-ol, were also detected in these incubations. In the aerobic KNO_3 plus glucose incubation only ethanol was detected, and in incuba-

Table 2. The effect of nutrient additives on the distribution by chemical functional groups of the volatile organic compounds (% total scan area) identified in the head space of aerobically incubated soil

Chemical group	Additive 0	KNO_3	KNO_3 + Glucose	$(NH_4)_2SO_4$	SED*
Alcohols	0.6	1.2	1.1	4.3	0.48
Ketones	3.9	18.6	6.2	17.8	3.44
Aldehydes	0.3	0.5	0.4	3.2	0.54
Esters	nd	nd	nd	0.3	NS
S-Compounds	75.7	73.1	79.1	66.1	NS
Aromatics	15.4	3.9	9.5	4.8	NS
Unidentified	4.1	2.8	3.6	3.6	NS

* Standard error of difference; nd = not detected, NS= not statistically different.

tions without N additions only ethanol and butan-2-ol were detected; these together with propan-2-ol were also produced when KNO_3 was added to the soil.

The proportions of aromatic compounds were not significantly different between incubations. Ethylbenzene, dimethyl benzene and benzene were detected in all the aerobic incubations, and the relative concentration of benzene was constant, at 1–2% of the total VOCs. The observed fluctuations in the relative proportions of total aromatic compounds were largely due to differences in the relative amounts of substituted benzenes, although trace quantities (<0.2% of total VOCs) of trimethyl benzenes, ethyl methyl benzenes and benzaldehyde were occasionally detected.

Esters were not found in the aerobic incubations, except for one replicate of the $(NH_4)_2SO_4$-enriched incubations, when butyl acetate was present as <1% of the total VOCs,

Anaerobic incubations

A greater proportion of the VOCs detected in the anaerobic incubations were alcohols. When soils were incubated with either added KNO_3 plus glucose or $(NH_4)_2SO_4$ (Table 3) alcohols accounted for almost 70% of the total VOCs. All 8 alcohols in Table 1 were detected in all the treatments, with the sole exception of soil incubated with KNO_3 plus glucose when butan-2-ol was not found. The predominant alcohol changed according to the N-supplementation (Fig.1). When no N was added 3-methyl butan-1-ol was the major alcohol produced, and the addition of KNO_3, KNO_3 plus glucose or $(NH_4)_2SO_4$ altered the predominant alcohol to ethanol, butan-1-ol or 2-methyl butan-1-ol respectively.

With the exception of pentan-3-one, all the monofunctional ketones listed in Table 1 were detected in all treatments. As in the aerobic incubations the major ketones were propan-2-one or butan-2-one with pentan-3-one only present in incubations with either no added N or added KNO_3. Also the bifunctional ketone, acetoin (3-hydroxybutan-2-one), previously reported as a volatile product of *Erwinia* infected potatoes was also detected in the KNO_3-enriched anaerobic soil incubations.

The amounts of aldehydes detected were not affected by any N-supplementation, and the major aldehyde produced was 3-methyl butan-1-al.

Dimethyl disulphide was again the main S-compound and, as in the aerobic samples, both the mono and trisulphides were found in all the anaerobic soil incubations. The proportion of sulphides was significantly affected by N-supplementation, with the levels found being in inverse proportion to the values found for the alcohols.

The same aromatic compounds were detected as in the aerobic incubations and the increases in total aromatic content in both the KNO_3 only and unsupplemented incubations were similarly due to an increase in the relative amounts of dimethyl benzenes.

Although there were no significant differences in total esters detected in the anaerobic incubations (Table 3), there were considerable differences in diversity. In particular, the $(NH_4)_2SO_4$-supplemented incubations yielded all the methyl and ethyl esters listed in Table 1, whilst in the KNO_3 plus glucose incubation all the ethyl and butyl esters were detected. In contrast the KNO_3-supplemented incubations yielded only ethyl acetate and in the unsupplemented samples only ethyl,

Table 3. The effect of nutrient additives on the distribution by chemical functional groups of the volatile organic compounds (% total scan area) identified in the head space of anaerobically fermented soil

Chemical group	Additive 0	KNO_3	KNO_3 + Glucose	$(NH_4)_2SO_4$	SED*
Alcohols	37.7	16.3	71.9	69.2	8.11
Ketones	13.3	21.1	8.5	6.2	2.37
Aldehydes	1.3	2.4	1.3	2.5	NS
Esters	0.2	0.4	6.2	3.9	NS
S-Compounds	24.2	23.2	7.3	11.7	2.95
Aromatics	23.3	28.7	2.9	6.2	6.54
Unidentified	nd	7.8	1.9	0.4	1.44

* Standard error of difference; nd = not detected, NS = not statistically different.

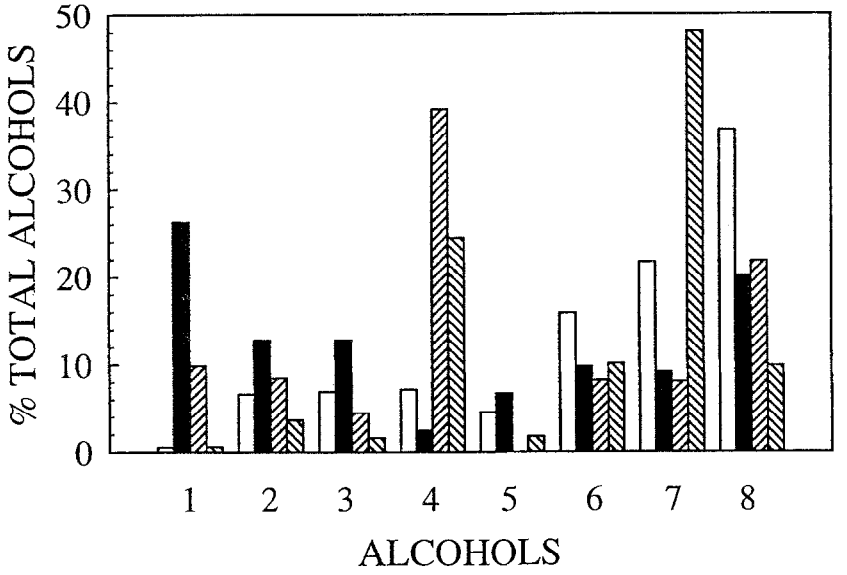

Figure 1. The alcohols produced during the anaerobic incubation of amended field soils. Amendments:-□, None; ■, KNO_3; ▨ KNO_3 plus Glucose; ▧, $(NH_4)_2SO_4$. Alcohols:- 1, ethanol; 2, propan-1-ol; 3, propan-2-ol; 4, butan-1-ol; 5, butan-2-ol; 6, methyl propan-1-ol; 7, 2-methyl butan-1-ol; 8, 3-methyl butan-1-ol.

2-methyl butanoate and methyl, 3-methyl butanoate were present.

Discussion

A greater diversity of VOCs was produced under anaerobic compared to aerobic conditions, 27 individual compounds were produced in the anaerobic incubation of soil compared with 13 in a similar aerobic incubation. Despite this wide diversity the VOCs produced in any particular incubation were consistent for those conditions. This consistent response to environmental conditions could also be particularly subtle, for example down to the single compound level in the alcohols. The responses to changes in aeration may well have been expected, but the responses to minor changes in N status, particularly of N-species probably would not.

These responses could be useful in monitoring specific microbial activities in situ, but it may well be more interesting to explore the possible role of the diverse range of VOCs identified here in communication between microorganisms in the rhizosphere, and the effects that this phenomenon may have on soil processes.

Consistency of production, both in terms of groups and individual compounds together with responsiveness to the environment, would be required if these

products are to act as signals between microbial groups, as this would allow for some constancy in action.

Dimethyl disulphide has been previously reported (Bremner and Bundy 1974; McCarty and Bremner 1991) to be an effective inhibitor of nitrification. Other compounds can also act as effective inhibitors of other processes. Fiddaman and Rosswall (1993) reported that a strain of *Bacillus subtilis* produced volatile compounds that severely impaired the growth of *Rhizoctonia solani* and *Pythium ultimum* . However, these effects can be variable. Ko and Hora (1972) reported that ammonia significantly reduced the germination rate of the conidia of *P. chrysogenum*, but when similar concentrations were combined with other volatiles the growth of some fungi such as *Fusarium solani* could be stimulated. Or indeed the effects can be selective. Schisler and Linderman (1989) demonstrated selectivity in action when they transferred volatiles between systems and showed that the VOCs produced by ectomycorrhizal Douglas Fir seedlings caused a significant increase in bacterial numbers, in receiver soils, but did not significantly affect populations of either actinomycetes, *Fusarium* sp., extracellular chitinase producers, facultative anaerobes or phosphate-solubilising bacteria. The same direct, but selective effect could also occur in cultivated soils. So it is possible that the rapid and widespread changes in the rates of specific microbial activities in the bulk soil may be a result of the VOCs produced by one part of the biomass influencing another, and this requires further investigation.

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